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LACTOGENIC EFFECTS OF TANNIN AND AGLYCONE FORM OF AQUEOUS EXTRACT (AFAE) FROM *Musa x paradisiaca* FLOWER ON LACTATING RATS

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ABSTRACT

This research is aimed to investigate the *in vivo* lactogenic effects of *Musa x paradisiaca* flower extracts, namely the aglycone form of aqueous extract (AFEA) and condensed tannin (CT) on lactating rats. Lactating rats (n=6) of Spraque Dawley with six pups each were orally administered with aqueous extract, AFEA, CT and metoclopramide. They were daily administered starting Day 5 until Day 14 and the performance was measured by weight-suckle-weight method. Results were analysed using SPSS by means of ANOVA at 0.05 and was expressed as their mean ± standard deviation. Milk production of rats treated with AFAE (4.65 ± 1.67 gm/pup/day) and aqueous extract (4.86 ± 2.36 gm/pup/day) were comparable with each other and significantly higher than negative control (3.63 ± 2.10 gm/pup/day) group. The groups treated with aqueous, AFAE, metoclopramide and CT were observed to increase milk yield by 28.03%, 24.87%, 2.63% and 2.20%, respectively, compared to negative control. The percentage body weight increment of pups for aqueous extract, AFAE, negative control, metoclopramide and CT, respectively were 148.21%, 122.29%, 113.8%, 101.53% and 87.06%. This research reveals the potential efficacy of bioactive compounds from *Musa x paradisiaca* flower to enhance milk production of inadequate supply of milk for breastfeeding mothers.

Key words: Lactogenic, Tannin, *Musa x paradisiaca*, Lactating rats

INTRODUCTION

Banana (*Musa* sp.) is one of the most important fruit and vegetable crops in many countries due to its enriched food and versatile medicinal values. Ethnobotanical studies proved that *Musa* sp. have been used in traditional folk medicine since thousands of years (Lans, 2006; Gachet *et al*., 2010). Almost all parts of the plant are valuable including leaf, fruit, stem, sap, flower and root to cure various ailments. Musa flowers are no exception to have tremendous pharmacological value based on ethnomedicinal survey around the world (Singh, 1986; Leonard, 1998). The flowers have been used traditionally to alleviate menorrhagia, dysentery, diabetes mellitus, asthma, hearth pain, stomach cramps and diarrhea (Iman & Akter, 2011; Sumathy *et al*., 2011).

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A few studies on Musa sp. have been associated its beneficial effect to women's health. The leaves of *Musa paradisiaca* L. were used in preparation of medicated water called *Vethuvellam* by boiling them in water and then used for bathing the mothers after delivery thus rejuvenate their body (Rajith *et al.*, 2010). Post-partum mother believed that the cool nature of banana plant as well as its fresh aroma in the mixture of preparation could interact the effects of the blood (Smith-Oka, 2008). Aspects of lactogenic plants have been studied extensively which include fenugreek, *Silybum marianum, Galega officinalis, Asparagus racemoses*, *Pimpinella anisum* L, *Nigella sativa*, *Cyperus rotundus* L, (Dog, 2009; Forinash *et al*., 2012; Badgujar & Bandivdekar, 2015; Hosseinzadeh *et al*., 2013; 2014).

Hundreds of plants have been screened for simulating effects on estrogen, oxytocin and other reproductive hormones. Estrogenic activity in

Asparagus racemosus (Shatavari) was reported due to hormone-like action of steroidal saponins (Sharma, 2011). The presence of steroidal saponins particularly sarsasapogenin and diosgenin in shatavari was considered to be partially associated with lactogenic effects (Behera *et al*., 2013). *Cyperus rotundus* L. was identified to increase milk production in lactating rats by causing development of breast tissue, increasing serum prolactin level, and increasing total protein and glycogen content in mammary gland (Badgujar & Bandivdekar, 2015). Meanwhile, *Cucurbita pepo* L was believed to support lactation by increasing serum prolactin level of lactating albino rats due to the presence of carbohydrate, glycosides, cardiac glycosides, saponin, tannin, alkaloid and flavonoids in the seed extract (Daniel *et al*., 2013).

There is no research reported concerning medicinal uses or pharmacological effects of banana flower means for galactagogue. Therefore, it is meaningful to search for alternative utilizations of the flower to make it more appreciable and valuable. Taking into consideration of the effectiveness of *Musa x paradisiaca* aqueous extract in promoting lactation (Mahmood *et al*., 2012), thus this study is aimed to evaluate further on the effects of Aglycone Form of Aqueous Extract (AFAE) and Condensed Tannin (CT) toward lactation in rats.

MATERIALS AND METHODS

Collection and preparation of plant materials

The flower of *Musa x paradisiaca* was obtained from cultivated local farmland in Jerantut, Pahang and was identified by a Botanish from the Institute of Biosiences, Universiti Putra Malaysia. The flowers were separated into florets and bracts, then dried in the oven for seven days at 40ºC. After dried, the samples were ground into powder and stored in air-tight container before extraction.

Extraction of aglycone form of aqueous extract (AFAE)

The glycoside part of the crude extract was removed using a method described by Ashnagar & Shiri (2011) with some modification. A sample of 500 gm was mixed with a solution of sodium acetate (80 mg) in water of 4 L. The mixture was continuously shaken in a water bath at 60ºC for about 4 hrs. After that it was filtered using double layers of muslin cloth. The filtrate was centrifuged at 3000 rpm for 10 min to get a clear supernatant. The solution was then freeze dried to get a dried powdered of 85.54 gm. The freeze dried sample was then hydrolyzed with 5% hydrochloric acid (HCl) and placed it on reflux for 14 hrs. The hydrolyzed mass was filtered and repeatedly washed with water until free from acid. The residue was later dried and extracted with n-hexane using soxhlet method for 4 hrs. The extract was concentrated using rotary evaporator to give a slightly yellow solid residue. The solid was finally purified with 95% ethanol and acetone to give white amorphous of 131 mg.

Extraction of condensed tannin (CT)

A sample of 200 g powdered dried sample was extracted with hexane for 12 hrs using a Soxhlet apparatus. The solvent was removed using rotary evaporator. Condensed tannins in the sample were then extracted twice at room temperature into 70% (v/v) aqueous acetone. The extracts were combined, evaporated to dryness under vacuum and then freeze dried to get a crude CT of 47.97 gm. The crude extract was purified according to the method described by Amarowicz *et al*. (2009). A sample of 1000 mg was suspended in 5 ml of 95% (v/v) ethanol and applied onto a chromatographic column packed with Sephadex LH-20 and equilibrated with 95% (v/v) ethanol. The column was exhaustively washed with 95% (v/v) ethanol and then eluted with 50% (v/v) acetone. Acetone elutes were combined and the solvent was removed under vacuum using rotary evaporator. The purified CT was freeze dried to get 623 mg of brown amorphous.

Animal test

All the experiments were carried out with Spraque Dawley rats purchased from the University Putra Malaysia. The rats were kept in a controlled room temperature (21 ± 2ºC) in a wood shaving cage, with adjusted lighting of 14 hrs lightness and 10 hrs darkness in a day. They were fed with commercial feeds (BARASTOC) and tap water *adlibitium* prior to and throughout the experiment. All the experiments were approved by the animal ethics committee of International Islamic University Malaysia.

Intervention procedure

Thirty female rats at age three months old with the weight of 200 – 350 gm were mated with male rats and were allowed to deliver their young. Each mother was adjusted to have only six pups per litter. The lactating rats composed of five groups (n=6) were given treatment with one of the following; aqueous extract, AFAE, CT, metoclopramide (positive control) or distilled water (negative control). The concentration of AFAE and CT in the dose given was consistent to the actual amount of each compound contains in the crude of the extracts. The amount of AFAE and CT administered to the rats were 0.765 mg/kg and 0.523 mg/kg, respectively. Positive control of rats were

administered with 0.2 mg/kg of metoclopramide. All the treatment groups were daily administered with samples started from Day 5 until Day 14 of lactation and data of milk productions were measured after 12 hrs of treatment starting from Day 6 until Day 15. Litters of the pup were isolated from their mothers for 6 hrs before milking. The weight of pups were measured twice before and after 60 min of milking to estimate the yield of milk. All the measurements of weight were read with accuracy of 0.01 gm using electronic balance (Mettler Toledo).

Statistical analysis

To determine whether the milk production were significantly different among treatment groups, data of different groups were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by LSD test ($p<0.05$) using SPSS for window.

RESULTS AND DISCUSSION

The rats treated with aqueous extract produced the highest yield, followed by AFAE, metoclopramide, CT and negative control (Table 1). Milk production of the rats treated with AFAE and aqueous extract were comparable with each other and significantly higher than negative control group. Meanwhile, the rats administered with CT showed no significant different in their milk production compared to the negative control group. The rats treated with aqueous extract showed the highest milk yield followed by AFAE, metoclopramide and CT.

The total amount of milk consumed by litters in one hr of milking during 10 days of lactation period showed the highest in the rats administered with aqueous extract followed by AFAE, metoclopramide, CT and negative control. The milk produced gradually increased by days of lactation until it reach at peak time, and a little bit decreased after the time (Figure 1). The mean of milk produced (gm/pup/day) in rats treated with aqueous and AFAE indicated constantly higher in yield along the experimental period except at Day 7 for aqueous which showed a bit decrease in yield.

The average body weight of the suckling pups on the starting day of data collection (Day 6) were almost equal in all the treatment groups. The pups weight at the final day (Day 15) of data collection also indicated no significant different in all the groups. The highest body weight increment of pups within 10 days of treatment was from the group administered with aqueous extract followed by AFAE, negative control, metoclopramide and CT (Table 2). Although, the final body weights of all treatment groups were comparable, but statistically their weight gain amongst the groups were significantly different. This means that, experimentally AFAE and CT were observed to react on the other way around. AFAE revealed its potential to increase lactation performance of mothers and growth rate of pups, while CT on the other hand reduced the performance and growth rate. AFAE and aqueous extract showed higher weight gain compared to CT and metoclopramide within ten days of experimental period ($p<0.05$).

Results of the present study showed that aqueous extract in its aglycone still has the same effects as in the form of its original crude. In other words, this finding revealed that removal of glycoside did not have an effect on milk production of lactating rats. Since there was no difference in the yield between AFAE and aqueous group, thus it is assumed that galactagogue effects should have come primarily from the AFAE constituent in which aglycone fraction contributed as the primary role in increasing milk production in this experiment. This finding is similar with those reported by some researchers in which although many compounds present with glycoside in their structure but only the non-conjugated (aglycone) forms appear to exert estrogen-like activity either in animals or human (Ye & Lou, 2005; Kang *et al*., 2012). Aglycones were also found to be better bioavailable due to increasing of body's efficiency to absorb and use of the compounds (Morand *et al*., 2000).

Table 1. Milk production of various treatments of extracts groups during 10 days lactation

Treatment group	Mean of milk production (gm/pup/day) ± S.D	Total milk production during 10 days (gm)	Quantity of milk at peak lactation time (gm/day)	Increment of milk produced (%)
Aqueous extract	4.86 ± 2.36[a]	279.1	43.5	28.03%
AFAE	4.65 ± 1.67[a]	272.2	32.7	24.87%
Metoclopramide	3.76 ± 2.25[b]	223.9	32.6	2.63%
CT	3.68 ± 2.15[b]	222.8	38.2	2.20%
Negative Control	3.63 ± 2.10[b]	218.0	31.5	–

Means followed by different superscript letters in the same column represent significant difference ($p < 0.05$).

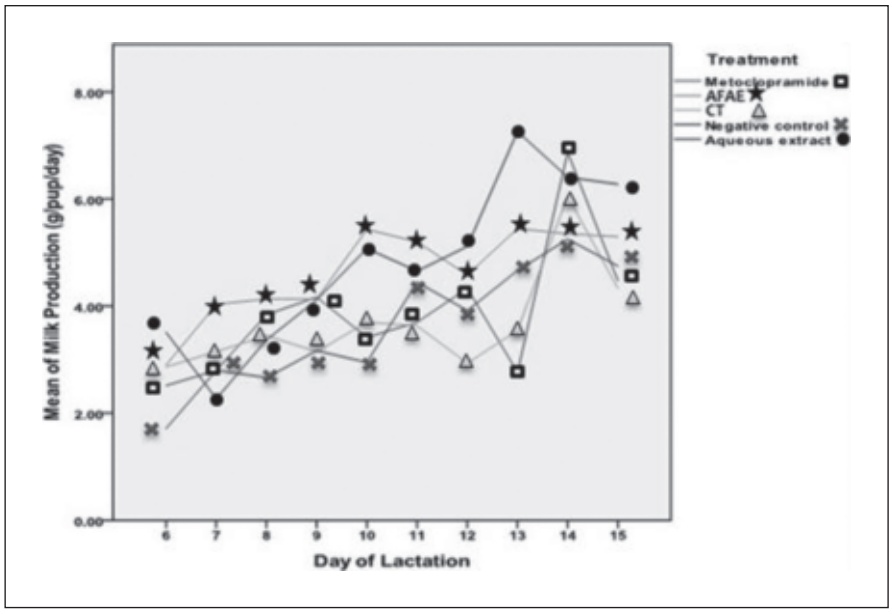

Fig. 1. Effect of treatment on milk production of lactating rats up to 15 days of lactation.

Table 2. Comparison between initial and final weight, percentage of body increment and weight gain of pups

Treatment	Mean of Initial weight (gm) ± SD	Mean of final weight (gm) ± SD	Percentage of BWI (%)	Weight gain (gm/pup) ± SD
Aqueous extract	11.20 ± 1.98[a]	27.80 ± 3.54[a]	148.21	1.85 ± 0.47[a]
AFAE	12.65 ± 1.43[a]	28.12 ± 1.17[a]	122.29	1.72 ± 0.35[a]
Negative control	11.23 ± 1.96[a]	24.01 ± 4.99[a]	113.8	1.42 ±0.51[ab]
Metoclopramide	11.77 ± 1.63[a]	23.72 ± 6.08[a]	101.53	1.36 ±0.71[b]
CT	12.83 ± 0.58[a]	24.00 ± 3.52[a]	87.06	1.28 ± 0.65[b]

Means followed by different superscript letters in the same column represent significant difference ($p < 0.05$). BWI = Body weight increment, SD = Standard Deviation.

It has clear in this research that the absent of CT in the extract would not affect the yield of milk in the rats treated with AFAE solely. Tannin is commonly known as anti-nutritional agents which consequently reduce digestibility of nutrients or in other way inhibits metabolic events (Kawas *et al*., 2010; Makkar, 2003). However, many studies proved that digestibility and nutrients were not affected by the condense tannin extract (Dschaak *et al*., 2011; Hymes-Fecht *et al*., 2005). Thus, we can say that the effects produced were not the same for all CT, but rather depend upon the concentration and structure of the CT.

Practically, the amount of metoclopramide administered to rats was in accordance to the dose suggested for the animals which was 0.2 mg/kg of its body weight. Thus, the dose given to rats was not the reason for no increment in the milk produced. It was reported that prolactin may reach 3-8 times the normal levels within 1 hr of metoclopramide administration and remain elevated for up to 8 hrs (Channa & Naqvi, 2013). Thus, the main reason why this research did not get significant milk production probably due to this limitation of the drug. This is because data was only collected daily after 22 hrs of administration of metoclopramide. This investigation showed that metoclopramide has shorter period of efficacy than *Musa* extracts, particularly the AFAE. The amount of milk produced was still far above the ordinary level in the AFAE group even though after 22 hrs of the intervention period. Thus, it clearly revealed that the efficacy to increase milk yield of AFAE as a natural plant extract was superior compared to metoclopramide, a modern synthetic drug.

CONCLUSION

Aglycon form of aqueous extract (AFAE) was identified to promote significant milk production compared to condensed tannin (CT). The efficiency

of the compound was identified as powerful and sustainable for a longer period than metoclopramide. The milk production in the rats treated with AFAE was equivalent to the amount produced in the aqueous extract group. This means that AFAE was the solely constituent responsible to the lactogenic activities and increase growth rate of the suckling pups. As the AFAE showed the strongest lactogenic activity, it was therefore suggested to perform in-depth investigation on isolated individual compound with the aim to identify specific components contain in the crude fraction, isolation and characterization of active compound(s). Finding of this research gave the idea for sustainability of underutilized banana flower as a natural alternative therapy for mothers who have inadequate milk supply.

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